

IN THE SPECIFICATION:

Please amend paragraph [0008] as follows:

[0008] The present invention features G-protein fusion receptors and chimeric GABA_B receptors (GABA_BRs), nucleic acid encoding such receptors, and the use of such receptors and nucleic acid. G-protein fusion receptors comprise at least one domain from a CaR, ~~a mGluR, an mGluR,~~ and/or a GABA_B receptor fused directly or through a linker to a guanine nucleotide-binding protein (G-protein). Chimeric GABA_BRs comprise at least one of a GABA_BR extracellular domain, a GABA_BR transmembrane domain, or a GABA_BR intracellular domain and one or more domains from a mGluR subtype 8 (mGluR8) and/or a CaR.

Please amend paragraph [0017] as follows:

[0017] The different receptor components of the G-protein receptor can come from the same receptor protein or from a chimeric receptor made up of different receptor domains. By swapping different domains, compounds able to ~~effect~~ affect different domains of a particular receptor can be identified and the activity of different compounds at different domains can be measured.

Please amend paragraph [0050] as follows:

[0050] FIGS. 9a-9p illustrate the cDNA sequence for human mGluR2 (SEQ. ID. NO. 26), chimeric ~~hCAR/hmGluR2- hCaR/hmGluR2~~ hCaR/hmGluR2 (SEQ. ID. NO. 30), chimeric hmGluR2/hCaR (SEQ. ID. NO. 34), and chimeric hmGluR8/hCaR (SEQ. ID. NO. 38).

Please amend paragraph [0051] as follows:

[0051] FIGS. 10a-10f illustrate the amino acid sequence for human mGluR2 (SEQ. ID. NO. 27), chimeric ~~hCAR/hmGluR2- hCaR/hmGluR2~~ hCaR/hmGluR2 (SEQ. ID. NO. 31), chimeric hmGluR2/hCaR (SEQ. ID. NO. 35), chimeric hmGluR8/hCaR (SEQ. ID. NO. 39).

Please amend paragraph [0054] as follows:

[0054] FIGS. 13a-13m illustrate the cDNA sequence for the ~~GABA-R2*Gqo5~~
GABA_BR2*Gqo5 fusion construct (SEQ. ID. NO. 42) and the ~~GABA-BR1a*Gqo5~~
GABA_BR1a*Gqo5 fusion construct (SEQ. ID. NO. 44).

Please amend paragraph [0055] as follows:

[0055] FIGS. 14a-14e illustrates the amino acid sequence for the ~~GABA-BR2*Gqo5~~
GABA_BR2*Gqo5 fusion construct (SEQ. ID. NO. 43) and the ~~GABA-BR1a*Gqo5~~
GABA_BR1a*Gqo5 fusion construct (SEQ. ID. NO. 45).

Please amend paragraph [0057] as follows:

[0057] FIGS. 16a-16e ~~illustrates~~ illustrate the amino acid sequence for the ph8SpmGluR4 chimeric construct (SEQ. ID. NO. 48), the amino sequence for the phmGluR4/CaR*AAA*G α qi5 fusion construct (SEQ. ID. NO. 49), and the phmGluR8//CaR*AAA* G α qi5 fusion construct (SEQ. ID. NO. 50).

Please amend paragraph [0064] as follows:

[0064] Eight distinct mGluR subtypes have been isolated. (Nakanishi, *Neuron* 13:1031, 1994; Pin and Duvoisin, *Neuropharmacology* 34:1, 1995; Knopfel et al., *J. Med. Chem.* 38:1417; *Eur. J. Neuroscience* 7:622-629, 1995, each of these references is hereby incorporated by reference herein.) The different mGluRs possess a large amino-terminal extracellular domain (ECD) followed by seven putative transmembrane-domain-domains (7TMD) comprising seven putative membrane-spanning helices connected by three intracellular and three extracellular loops, and an intracellular carboxy-terminal domain of variable length (cytoplasmic tail).

Please amend paragraph [0065] as follows:

[0065] Human mGluR8 is described by Stormann *et al.*, U.S. Patent Nos. 6,051,688, 6,077,675, and 6,084,084, International Application Number No. PCT/US97/09025, International Publication Number No. WO 97/48724, and mouse mGluR8 is described by

Duvoisin et al., J. Neurosci. 15:3075-3083, 1995 (both of these references are hereby incorporated by reference herein). mGluR8 couples to G_i. Agonists of mGluR8 include L-glutamate and L-2-amino-4-phosphonobutyrate.

Please amend paragraph [0068] as follows:

[0068] The CaR responds to changes of extracellular calcium concentration and also responds to other divalent and trivalent cations. The CaR is a G-protein-coupled receptor containing an extracellular Ca²⁺ binding domain. Activation of the CaR, descriptions of CaRs isolated from different sources, and examples of CaR active compound are provided in Nemeth *NIPS* 10:1-5, 1995; Brown *et al.* U.S. Patent No. 5,688,938; Van Wagenen *et al.*, International Application-Number PCT/US97/05558-No. PCT/US97/05558, International Publication-Number No. WO 97/37967; Brown E.M. *et al.*, *Nature* 366:575, 1993; Riccardi D., *et al.*, *Proc. Nat'l. Acad. Sci. USA* 92:131-135, 1995; and Garrett J.E., *et al.*, *J. Biol. Chem.* 31:12919-12925, 1995. (Each of these references ~~are~~ is hereby incorporated by reference herein.) Brown *et al.* U.S. Patent No. 5,688,938, and Van Wagenen *et al.*, International Application-Number PCT/US97/05558-No. PCT/US97/05558, International Publication-Number No. WO 97/37967, describe different types of compounds active at the CaR including compounds that appear to be allosteric modulators and CaR antagonists.

Please amend paragraph [0069] as follows:

[0069] The CaR can be targeted to achieve therapeutic effects. Examples of target diseases are provided in Brown *et al.* U.S. Patent No. 5,688,938, and Van Wagenen *et al.*, International Application-Number PCT/US97/05558-No. PCT/US97/05558, International Publication-Number No. WO 97/37967, and include hyperparathyroidism and osteoporosis.

Please amend paragraph [0072] as follows:

[0072] Different GABA_BR subtypes exist. Kaupmann *et al.*, *Nature* 386:239-246, 1997, indicate that they cloned GABA_BRs. Nucleic acid encoding two GABA_BR proteins were indicated to be cloned from rat brain: GABA_BR1a and GABA_BR1b. GABA_BR1a differs from

GABA_BR1b in that the N-terminal 147 residues are replaced by 18 amino acids. GABA_BR1a and GABA_BR1b appear to be splice variants. The cloned GABA_BRs were indicated to negatively couple adenylyl cyclases and show sequence similarity to the metabotropic receptors for L-glutamate (mGluR). Northern blot analysis indicated that GABA_BR1a and GABA_BR1b are present in brain and ~~testis~~, testes, but not in kidney, skeletal muscle, liver, lung, spleen, or heart.

Please amend paragraph [0073] as follows:

[0073] Kaupmann *et al.*, International Application ~~Number~~ No. PCT/EP97/01370, International Publication ~~Number~~ No. WO 97/46675, indicate that they have obtained rat GABA_BR clones, GABA_BR1a and GABA_BR1b; and human GABA_BR clones, GABA_BR1a/b (representing a partial receptor clone) and GABA_BR1b (representing a full-length receptor clone). Amino acid sequence information and encoding cDNA sequence information are provided for the different GABA_BR clones.

Please amend paragraph [0076] as follows:

[0076] GABA_BRs have been targeted to achieve therapeutic effects. Kerr and Ong, *DDT* 1:371-380, 1996, describe different compounds indicated to be GABA_BR agonists and GABA_BR antagonists. Kerr and Ong also review therapeutic implications of affecting GABA_BR activity including, spasticity and motor control, analgesia, epilepsy, cognitive effects, psychiatric disorders, alcohol dependence and withdrawal, feeding behavior, cardiovascular and respiratory functions, and peripheral functions.

Please amend paragraph [0077] as follows:

[0077] Bittiger *et al.*, *Tips-TIPS* 4:391-394, 1993, review therapeutic applications of GABA_BR antagonists. Potential therapeutic applications noted by Bittiger *et al.* include cognitive processes, epilepsy, and depression.

Please amend paragraph [0090] as follows:

[0090] A variety of different activities have been generally attributed to GABA_BR subtypes. (E.g., Kerr and Ong, DDT 1:371-380, 1996.) Kaupmann *et al.*, *Nature* 386:239-246, 1997, report that in preliminary experiments involving GABA_BR1a, they did not detect positive coupling to the adenylyl cyclase or coupling to the phospholipase effector system.

Please amend paragraph [00102] as follows:

[00102] While the effect of an amino acid change varies depending upon ~~factors~~ factors, such as phosphorylation, glycosylation, intra-chain linkages, tertiary structure, and the role of the amino acid in the active site or a possible allosteric site, it is generally preferred that a substituted amino acid is from the same group as the amino acid being replaced. To some extent, the following groups contain amino acids that are interchangeable: the basic amino acids lysine, arginine, and histidine; the acidic amino acids aspartic and glutamic acids; the neutral polar amino acids serine, threonine, cysteine, glutamine, asparagine and, to a lesser extent, methionine; the nonpolar aliphatic amino acids glycine, alanine, valine, isoleucine, and leucine (however, because of size, glycine and alanine are more closely related and valine, isoleucine and leucine are more closely related); and the aromatic amino acids phenylalanine, tryptophan, and tyrosine. In addition, although classified in different categories, alanine, glycine, and serine seem to be interchangeable to some extent, and cysteine additionally fits into this group, or may be classified with the polar neutral amino acids.

Please amend paragraph [00122] as follows:

[00122] Pharmaceutically acceptable salts include acid addition ~~salts~~ salts, such as those containing sulfate, hydrochloride, fumarate, maleate, phosphate, sulfamate, acetate, citrate, lactate, tartrate, methanesulfonate, ethanesulfonate, benzenesulfonate, *p*-toluene-sulfonate, cyclohexylsulfamate and quinate.

Please amend paragraph [00123] as follows:

[00123] Pharmaceutically acceptable salts can be obtained from ~~acids~~ acids, such as hydrochloric acid, maleic acid, sulfuric acid, phosphoric acid, sulfamic acid, acetic acid, citric acid, lactic acid, tartaric acid, malonic acid, methanesulfonic acid, ethanesulfonic acid, benzenesulfonic acid, *p*-toluenesulfonic acid, cyclohexylsulfamic acid, fumaric acid, and quinic acid.

Please amend paragraph [00124] as follows:

[00124] Pharmaceutically acceptable salts also include basic addition ~~salts~~ salts, such as those containing benzathine, chloroprocaine, choline, diethanolamine, ethylenediamine, meglumine, procaine, aluminum, calcium, lithium, magnesium, potassium, sodium, ammonium, alkylamine, and zinc, when acidic functional groups, such as carboxylic acid or phenol are present. For example, see *Remington's Pharmaceutical Sciences* 18th ed., Mack Publishing Co., Easton, PA, p. 1445, 1990. Such salts can be prepared using the appropriate corresponding bases.

Please amend paragraph [00139] as follows:

[00139] In a separate PCR reaction using phmGluR4 as template, a 472 bp fragment of human mGluR4 was amplified using a hybrid primer 4/8RP (sense 42-mer, exactly ~~complimentary~~ complementary to primer 8/4RP) and oligo mG4-472R (antisense 18-mer, complementary to the human mGluR4 cDNA; sequence 5'-ctgaaggccggatgacae-3') (SEQ ID NO:53). The two PCR products generated from the above two reactions were annealed together in equimolar ratios in the presence of the external primers mG4-472R and 3.1-535F, and Turbo Pfu DNA polymerase (Strategene).

Please amend paragraph [00151] as follows:

[00151] The first reaction used two primers, 2-1713 (sense 21-mer, corresponding to nucleotides 1710-1730 of human mGluR2, Genbank Accession # 4504136) and the hybrid primer 2/CT (antisense 42-mer, containing 21 nucleotides complementary to nucleotides

2452-2472 of human mGluR2 and 21 nucleotides complementary to nucleotides 2602-2622 of the human CaR). These primers were used to amplify a 783 bp PCR fragment of human mGluR2. In a separate PCR reaction using phCaR in the BlueScript SK⁻ plasmid-as-as a template, a 750 bp fragment of the human CaR was amplified using a hybrid primer CT/2 (sense 42-mer, exactly complementary to primer 2/CT) and the T3 primer commercially available from Stratagene.

Please amend paragraph [00167] as follows:

[00167] The first reaction used two primers, CH5A (sense 19-mer, corresponding to nucleotides 2187-2205 of human mGluR8, Stormann *et al.*, U.S. Patent Nos. 6,051,688, 6,077,675, and 6,084,084, and International Publication Number No. WO97/48724) and the hybrid primer CH5B (antisense 40-mer, containing 22 nucleotides complementary to nucleotides 2523-2544 of human mGluR8, and 18 nucleotides complementary to nucleotides 2602-2619 of the human CaR). These primers were used to amplify a 375 bp PCR fragment of human mGluR8. In a separate PCR reaction using phCaR in the BlueScript SK(-) plasmid-as-as a template, a 750 bp fragment of the human CaR was amplified using a hybrid primer CH5C (sense 40-mer, exactly complementary to primer CH5B) and the T3 primer commercially available from Stratagene.

Please amend paragraph [00178] as follows:

[00178] Test substances were applied by superfusion at a flow rate of about 5 ml/minute. Receptor fusion activation was determined by measuring the increase in calcium-activated chloride current (I_{Cl}). Increases in I_{Cl} were quantified by measuring the peak inward current stimulated by activating agent, relative to the holding current at -60 mV. Application of 100 μ M L-glutamate elicited a response from the mGluR2//CaR*G_{q/11} and mGluR8//CaR*G_{q/11}. Application of 100 μ M Gd³⁺ activated the CaR/mGluR2*G_{q/11}.